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Mutations in rDNA. 1. Dependence of Chromosome Mutation Induction on Positions and Activity of Nucleolus Organizer Regions

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Summary. Studies on chromatid aberration induction in NORs of standard and reconstructed karyotypes, as well as in the single translocation lines of barley, indicate a correlation between synthetic activity (transcription) of rDNA and frequency of chromosome mutations induced by HU. Experimental evidence in favour of this inference arises from analyzing karyotypes with NORs located at their original sites and karyotypes with NORs translocated from their original sites. The close correlation between the different rate of synthetic activity (transcription) in nucleolus formation and the comparable range of variation in aberration involvement of NORs observed in translocation lines are discussed.

Key words: NOR – Transcription – Chromatid aberrations – Barley – HU – Reconstructed karyotype

Introduction

Nucleolus organizer regions (NORs), which are associated with secondary constrictions of satellite chromosomes, have been shown to be the sites of the DNA (rDNA) complementary to the 25-28S and 18S ribosomal RNA (rRNA) in *Drosophila* (Ritossa and Spiegelman 1965), *Xenopus* (Wallace and Birnstiel 1966; Birnstiel et al. 1971), maize (Phyllips et al. 1974), *Vicia faba* (Scheuerman and Knälmann 1975) and *Hordeum* (Subrahmanyam and Gerlach 1978; Subrahmanyam and Azad 1978). In eukaryotes, the ribosomal DNA (rDNA) consists of multiple rDNA cistrons which are clustered tandemly at the NORs (Attardi and Amaldi 1970); Birnstiel et al. 1971) coding for ribosomal RNA molecules, i.e. 25 to 28S rRNA, 18S rRNA and 5,8S rRNA. The number of rRNA cistrons differ within the eukaryotes, ranging from 250 copies in *Drosophila* (Ritossa and Spiegelman 1965) to 300,000 copies in some plant species (Ingle and Sinclair 1972; Hotta and Miksche 1974; Subrahmanyam and Azad 1978).

Each of the repeating units (18S-5,8S-25-28S) in the NORs involves one coding sequence for each of the RNAs. These alternate with transcribed and non-transcribed spacer DNA sequences and are transcribed as a single common precursor rRNA molecule (primary transcript) which is ultimately larger than the sum of the final molecules into which the precursor molecule becomes stepwise processed (Perry 1976; Hadjiolov and Nikolaev 1976). For review see also Reeder (1974). The difference in length of the repeating unit has been described in *Drosophila* (Tartof and David 1976), mouse (Cory and Adams 1977) and wheat, rye and barley (Appels et al. 1980). This repeat unit heterogeneity has been detected e.g. in spacer regions of the two different repeats (9,5 kb and 10 kb) found in barley, and within the 26S coding sequences in wheat and rye (Appels et al. 1980). It may also be connected with an insert sequence of variable length within the 28S gene of *Drosophila* (Glover and Hogness 1977; Pellegrini et al. 1977). The primary gene products (rRNA), their associated proteins (ribosomal proteins) and the enzymatic equipment (RNA polymerase I, RNA methylase(s), RNA processing enzyme(s)) necessary for synthesis, conversion and assembly of the ribosomes, involve the nucleolus (Birnstiel et al. 1971; Smetana and Bush 1974; Perry 1976; Hadjiolov and Nikolaev 1976).

Electron-microscopic studies (Lafontaine and Chouinard 1963; Brinkley 1965; Royal and Simard 1975; Goessens 1976; Jordan and Luck 1976) demonstrated that the secondary constrictions of metaphase chromosomes do not differ ultrastructurally from the fibrillar centers of the interphase nucleolus which have been found to synthesize RNA. Miller and Beaty (1969), Spring et al. (1974) and Angelier and Lacroix (1975) visualized the transcription activity of rDNA within the fibrillar centers.

From the findings presented it is evident that NORs represent small and clearly defined portions of the eukaryotic karyotype, which with respect to analyzing mutation expression and specificity of the rDNA, have the main advantage that rDNA is their only active gene.

'Nucleolar mutations' usually involve changes in the number of rRNA cistrons (deletions), the activity of NORs (nucleolar dominance and position effects) and structural chromosomal rearrangements.

Well-known cases of deletion mutants are the anucleate mutations in *Xenopus laevis* which have been shown to consist of complete deletions of the rDNA (Wallace and Birnstiel 1966), and a variety of mutants of *Xenopus laevis* with only partial deletions

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in the rDNA (Miller and Knowland 1970, 1972; Knowland and Miller 1970). In a mutant found by Humphrey (1961) in *Axolotl*, the formation of abnormally small nucleoli has also been shown to be a partial deletion of the rDNA (Miller and Brown 1969). Mutants due to deletions of rDNA have also been described in *Chironomus* (Pelling and Beermann 1966), *Sciara* (Gerbi 1971) and *Drosophila* (Ritossa et al. 1966; Weinman 1972; Schermoen and Kiefer 1975), in which partial deletion of the rDNA is correlated with 'bobbed' phenotype.

Another well-known type of alteration in rDNA concerning NOR activity without any change in the number of rRNA cistrons are the effects of the nucleolar dominance (differential amphiplasty) (Nawashin 1928, 1934) in interspecific hybrids and the position effects in some translocation barley lines.

Nucleolar dominance has been observed in interspecific plant and animal hybrids and is made evident by the partial or complete repression of transcription of ribosomal RNA of one parent by the other. Due to different genome interaction this effect is completely reversible when the interacting genomes are separated from each other (for review see Rieger et al. 1979). Analogous effects have been observed in some barley translocation lines where the transcription of NORs translocated to new positions in the chromosome complement by interchanges has been studied (Nicoloff et al. 1977 a, b, 1979; Anastassova-Kristeva et al. 1977, 1979). The results obtained from these lines, showed that the synthetic activity of the rRNA cistrons becomes characteristically impaired when all NORs are combined in a single chromosome pair.

In connection with structural rearrangements involving the NORs, Kihlman et al. (1966) have reported that when meristem cells of *Vicia faba* treated with hydroxyurea were recovered after 16-24 hours in a hydroxyurea-free solution and then fixed, a high proportion (more than 50%) of the chromatid exchanges obtained were localized in the nucleolar constriction, i.e. in the NOR. Recently it has been shown that the secondary constriction of *Vicia faba* is only one out of several chromosome segments which demonstrated partial mutagen-specific aberration clustering, i.e. chromosome segments which are involved in chromatid aberrations significantly more than expected on the basis of their relative metaphase length (Rieger et al. 1975; Schubert and Rieger 1976; Slotova et al. 1977; Rieger et al. 1977). However, the secondary constriction of the same species was found to be the only site of the chromosome complement involved in sister chromatid exchanges (SCE), both without and with mutagen treatment, more frequently than expected on the basis of its relative metaphase length (Schweizer 1973; Schubert et al. 1980). In *H. vulgare* however, the NORs are not SCE hot spots (Schubert et al. 1980).

Additionally, it is also evident that satellite chromosomes with active NORs are significantly over-represented in Robertsonian translocations in mouse cell lines. This indicates that the nucleolus plays an important role in the origination of these translocations (Miller et al 1978). Although the activities of the NORs in different mutants and the induction of chromosome mutations in NORs after treatment with mutagens have both been extensively studied, these two approaches have not yet been adequately integrated.

In the present paper we report on indications of a dependence of the frequency of chromatid aberrations in NORs upon transcriptional activity of rDNA in different (standard and mutant) lines of barley.

Materials and Methods

As mentioned above, a number of studies have revealed some chemical compounds which induce a relatively high proportion (more than 50%) of chromatid exchanges localized in the nucleolar constriction of *Vicia faba*. These include hydroxyurea (HU) (Kihlman et al. 1966) 2'-deoxyadenosine (2'-AdR) (Kihlman 1955, 1961; Kihlman and Odmark 1965) and 5-fluoro-2'-deoxyuridine (FudR) (Kihlman 1962). It was considered desirable to learn whether this holds also true for nucleolar constrictions of *Hordeum vulgare*. Under the treatment conditions applied in the present study, it becomes clear that the effect of HU on *Hordeum vulgare* was quite similar. Thus, the use of such chemicals (HU) with a comparatively specific action on the NOR in inducing structural chromosome mutations, provides the manner to develop a real program for studying the relationship between transcriptional activity and mutagenic expression of rDNA.

HU is a potent inhibitor of DNA synthesis but has an insignificant effect on the synthesis of RNA (Young and Hodas 1964; Rosenkranz et al. 1966). The mechanism by which it inhibits DNA synthesis is thought to be due to an interference with ribonucleotide reduction. The effect on DNA synthesis can be easily reversed by the removal of HU or by the addition of deoxyribonucleosides (Young and Hodas 1964; Rosenkranz et al. 1966; Adams and Lindsay 1967; Brockman et al. 1970). Ben-Hur and Ben-Jshai (1971) reported that following UV irradiation, HU caused an inhibition of strand rejoining, the final stage of the DNA repair process. However, the data obtained by Clarkson (1978) confirmed that HU has a detrimental effect on DNA repair although he did find that 'this does not appear to be due to inhibition of repair synthesis, but rather by increasing it so that ligation is prevented, or by preventing ligation with the result that excision and resynthesis continue in uncontrolled manner'.

In the present study a standard karyotype (var. 'Frigga'), a reconstructed karyotype (MK 14/2034), and five single translocation lines (T 21, T 505, T 506, Td 130 and Td 412) of *Hordeum vulgare* have been treated with HU and analysed for their response to this agent. The chromosome complement of the standard karyotype contains two pairs of satellite chromosomes: one (pair 6) with a longer satellite, the other (pair 7) with a shorter satellite. The reconstructed barley karyotype (MK 14/2034) was originated by means of reciprocal translocations which involved chromosomes 1 and 7 of the standard complement of var. 'Elgina' and chromosomes 3 and 4 of the standard complement of var. 'Frigga' (Künzel and Nicoloff 1979). All chromosome pairs in this karyotype are morphologically distinguishable. The four single translocation lines have all four NORs combined either in opposite arms of a single satellite chromosome 6^7 (T 505) or in chromosome 7^6 (T 506) (Nicoloff et al. 1977, 1979) or tandemly in one chromosome arm of chromosome 6^7 (Td 412) or chromosome 7^6 (Td 130) (Hagberg et al. 1978; Hagberg and Hagberg 1978). Each of the two homologous satellite chromosomes in these karyotypes, therefore, contains two secondary constrictions and two satellites. The single translocation line T 21 differs from the standard karyotype of barley by the presence of three chromosomes (5^7, 6 and 7^5) instead of two chromosomes pairs with nucleolus organizer activity. This is due to an interchange with translocation points localized in the short arm of chromosome 5 and within the secondary constriction of the satellite chromosome 7 close to the satellite (Anastassova-Kristeva et al. 1977; Nicoloff et al. 1977).

For the localization of aberrations, the standard and reconstructed karyotypes were subdivided into 48 segments (Nicoloff et al. 1975; Nicoloff and Künzel 1976; Künzel and Nicoloff 1979), whose individual involvement in aberrations was tested by use of reconstructed karyotype MK 14/2034 (Fig. 1). The individual involvement of segments in the satellite chromosomes was tested only in the standard karyotype and the translocation karyotypes (T 505, T 506, Td 130 and Td 412). Four types of induced chromatid aberrations were scored and located for each segment: isolocus

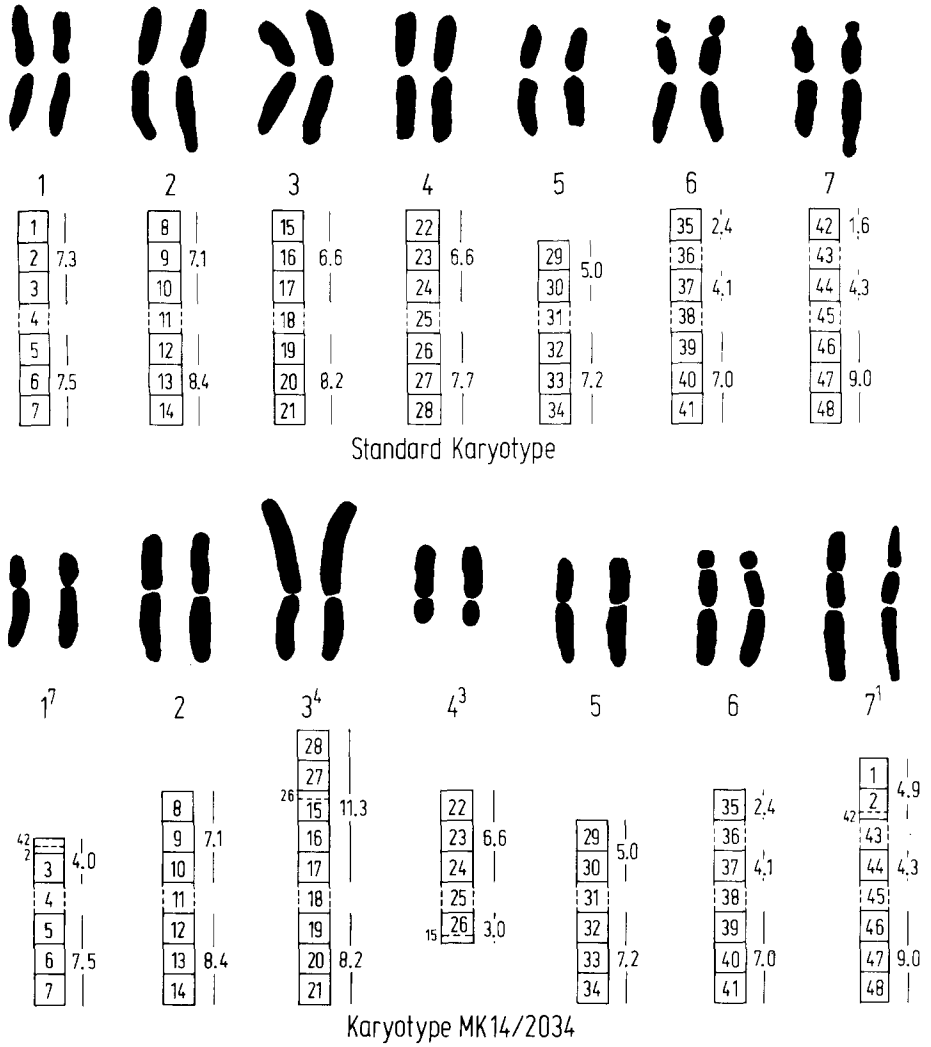

Fig. 1. Standard karyotype and reconstructed karyotype MK 14/2034 of barley

breaks (i), duplication-deletions (dd), intercalary deletions (d) and chromatid translocations (t). The total number of isolocus breaks, duplication-deletions, intercalary deletions and lesions involved in chromatid translocations was taken as 100% and the percentage involvement of the individual segments in these aberration types respectively presented. Confidence limits (α = 1%) were calculated for the segments.

Presoaked seeds (1 h in tap water), kept on moist filter paper for 18 h in an aerated desiccator (24°C), were treated with HU (4 or 6 h, 3.10^{-3} M, 24°C). After treatment, seeds were germinated in Petri dishes (24°C) and fixed (1:3 glacial acetic acid and ethanol) after periods (recovery times) of 12, 15, 18, 21, 24 and 27 h. Before fixation, the germinated seeds were immersed (2 h) in a solution of 0.025% colchicine saturated with α-bromo-naphtalene. Following maceration of the roots in pectinase (4% for 30 min) permanent Feulgen squashes were prepared.

Results

Karyotypes with NORs Located at their Original Sites

Three karyotypes (reconstructed karyotype MK 14/2034, standard karyotype and translocated karyotype T 21) having all four NORs located at the original sites of the four satellite chromosomes 6 and 7 were studied.

The pattern of intrachromosomal distribution of chromatid abberrations induced after treatment of the reconstructed barley karyotype (MK 14/2034) with HU is shown in Fig. 2. The distribution pattern of chromatid aberrations in this karyotype is characterized by a comparatively low aberration clustering in the 'common' segments. There is, however, marked preferential involvement in chromatid translocations of segment 36 (NOR of chromosome 6 – about 11%) and segment 43 (NOR of chromosome 7 – about 16%). The bulk of chromatid translocations is therefore clustered mainly in these two segments. The percentage involvement in aberrations (i + t) of segments 36 and 43 after treatment with HU is about 40.1 as compared to the total involvement in aberrations of all segments investigated (100%) in this karyotype. This means that a comparatively high proportion of induced

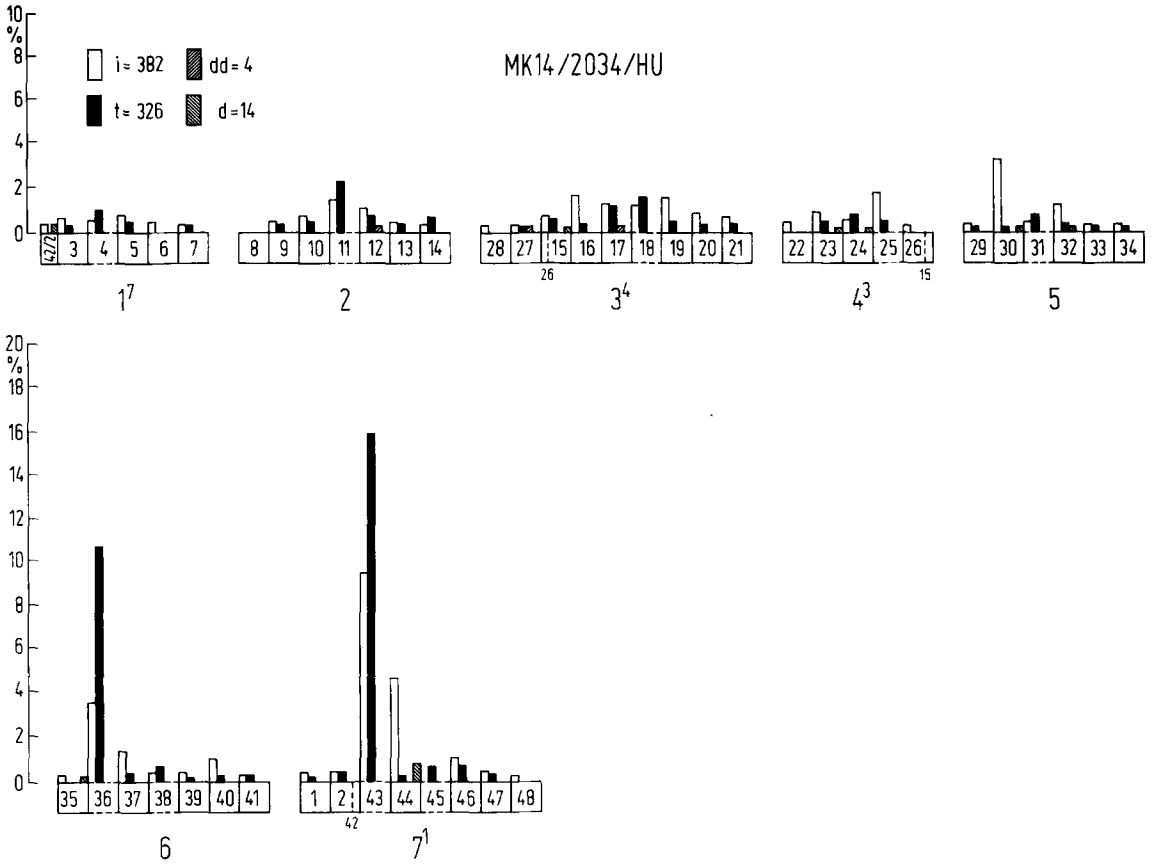

Fig. 2. The percent involvement in chromatid aberrations (i = isochromatid breaks; dd = duplication-deletions; d = deletions; t = lesions involved in chromatid translocations) of chromosomes (reconstructed karyotype MK/2034) subdivided into 48 segments after treatment with hydroxyurea (HU)

chromatid aberrations is preferentially localized in the nucleolus organizer regions. The frequences of the translocations in segments 36 and 43 only surpassed the upper limit of confidence intervals (21).

Similar translocation clustering has also been observed in the standard karyotype (about 12% in segment 36 and 18% in segment 43) (Fig. 3) as well as in translocation karyotype T 21 (about 13% in segment 36 and 20% in segment 43) (Fig. 4). In the standard karyotype the involvement of segment 36 and 43 in aberrations (i + t) is about 34,2% and in karyotype T 21, about 41.7%. The frequency of the translocations in segments 36 and 43 surpassed the upper limits of confidence intervals (26) for the standard karyotype and 15 for karyotype T 21).

The most striking feature which emerges from the data obtained in these karyotypes is that the frequencies of chromatid translocations clustered in segment 43 tend to be higher than these in segment 36. The difference in the frequencies invariably ranges from 5 to 7%.

Although the number of rRNA cistrons is reduced in the NOR (segment 43^{30}) of chromosome 7^{5} in karyotype T 21, the percentage involvement in translocations of this

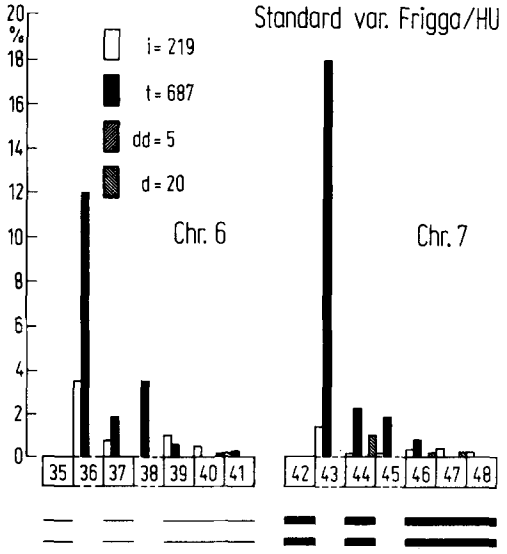

Fig. 3. Translocation clustering in segments 36 (NOR of chromosome 6) and 43 (NOR of chromosome 7) after treatment with hydroxyurea of standard karyotype (var. 'Frigga')

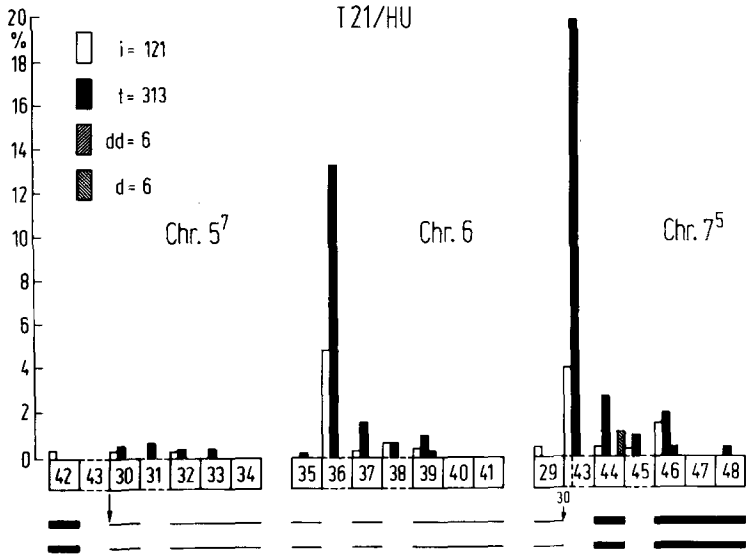

Fig. 4. Translocation clustering in segments 36 (NOR of chromosome 6) and 43^{30} (NOR of chromosome 7) after treatment with hydroxyurea of translocated karyotype T 21. In this karyotype a portion of the NOR of standard chromosome 7 (break point in NOR) is interchanged with a part of the short arm of chromosome 5. Satellite chromosome 6 is unchanged. Arrows = translocation points

segment remains unchanged, i.e. is practically the same as in the standard karyotype.

Of great interest is also the fact that segment 43, which represents part of the secondary constriction of satellite chromosome 7 translocated to chromosome 5 (5^7) in the translocated karyotype T 21, is not involved in any breaks or translocations (Fig. 4).

Karyotypes with NORs Translocated from Their Original Sites

Four translocation lines (T 505, T 506, Td 130, and Td 412) with all four NORs combined in a single chromosome pair, either in opposite arms of satellite chromosomes 6 or 7 (6^7, 7^6) (T 505 and T 506) or tandemly in one chromosome arm of satellite chromosomes 6 or 7 (6^7, 7^6) (Td 130 and Td 412), were studied with respect to their involvement in aberrations of segments 36 and 43 (NORs of chromosome 6 and 7, respectively).

In karyotype T 505, the NOR, together with the satellite of chromosome 6 (segment 36 and 35), were translocated from their original sites to the long arm of satellite chromosome 7 (7^6) (Fig. 5).

The percentage involvement in aberrations (i + t) of segments 36 and 43 is not more than 19.3 in this structurally changed karyotype, the highest proportion of aberrations being localized in the 'common' chromosome segments, but not in segments 36 and 43, as it is observed in the standard karyotype. As seen from Figure 5, the frequency of the chromatid exchanges in segment 43 decreased to about 6%, indicating that this segment still remains a translocation hot spot. The frequency of translocations in segment 43 did surpass the upper limit of confidence intervals (30).

A similar effect after treatment with HU has also been observed in translocation karyotype T 506. In this karyotype, the NORs with satellites of chromosome 7 (segments 43 and 42) are translocated from their original sites to the long arm of satellite chromosome 6 (6^7). This also resulted in one homologous pair of satellite chromo-

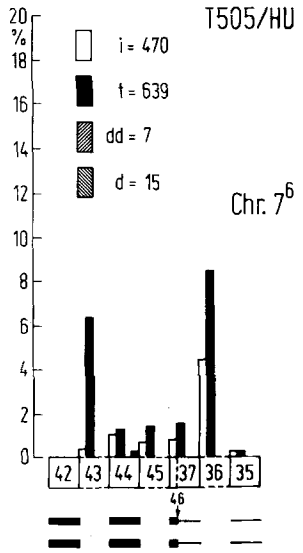

Fig. 5. Chromosome 7^6 of translocation karyotype T 505. As a result of translocation of NOR and satellite of chromosome 6 (segments 36 and 35) from their original site to long arm of satellite chromosome 7, two NORs are combined in opposite arms of this chromosome

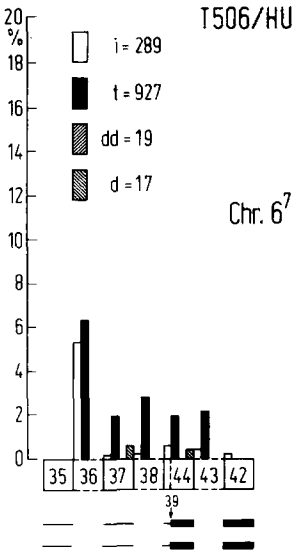

Fig. 6. Chromosome 6^7 of translocation karyotype T 506. Due to interchanges of NOR and satellite of chromosome 7 (segments 43 and 42) from their original site to the long arm of chromosome 6, two NORs are combined in opposite arms of this chromosome

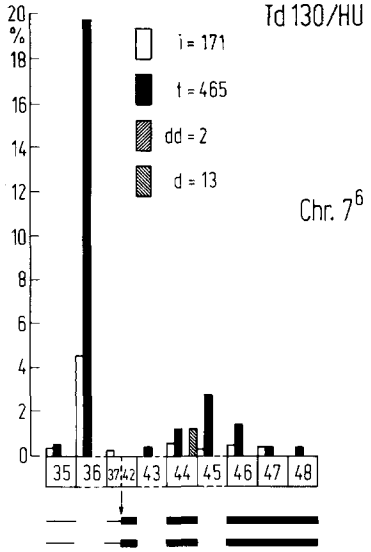

Fig. 7. Chromosome 7^6 of translocation karyotype Td 130. The translocation of NOR and satellite of chromosome 6 (segments 36 and 37) from their original site to satellite of chromosome 7 resulted in two NORs combined tandemly in this chromosome

somes (6^7) with all four NORs in opposite arms (Fig. 6).

In this karyotype the percentage involvement in aberrations (i + t) of segments 36 and 43 is comparatively much lower (about 14.1) with most aberrations being localized in other chromosome segments, but not in segments 36 and 43.

The frequency of breaks and translocations localized in segment 36 ranges up to 6% although this frequency is not higher than 2% in segment 43, which means that this segment is no longer a translocation hot spot after treatment with HU (Fig. 6). The frequency of the translocations in segment 43 did not surpass the upper limit of the confidence intervals (33).

In karyotype Td 130, the NOR of satellite chromosome 6 with the satellite (segments 36 and 35), is transposed to the satellite of chromosome 7 resulting in a translocation chromosome 7^6 with two NORs tandemly arranged in its short arm. The percentage involvement in breaks and translocations of segments 36 and 43 (NORs of chromosome 6 and 7 respectively) is again lower in this karyotype (about 27%), as compared to the standard karyotype. There is, however, a significant difference in the translocation involvement of segments 36 and 43, since segment 36 shows a markedly higher clustering of translocations (about 20%), while the involvement in translocations of segment 43 is practically insignificant (about 0.4%). This segment is therefore no longer a translocation hot spot (Fig. 7). The frequency of the translocations in segment 43 did not surpass the upper limit of confidence intervals (20).

In the last karyotype (Td 412) analyzed, the nucleolus organizer region (segment 43) with the satellite (segment 42) of chromosome 7 are translocated from their original sites to the satellite of chromosome 6; this also results in all four NORs being tandemly arranged in only one homologous pair of satellite chromosomes (6^7). In this case the percentage involvement in aberrations of segments 36 and 43 is approximately one half (about 18.7) of that in the standard karyotype positions. The aberration frequency in segment 43 is rather low (about 2%) and this seg-

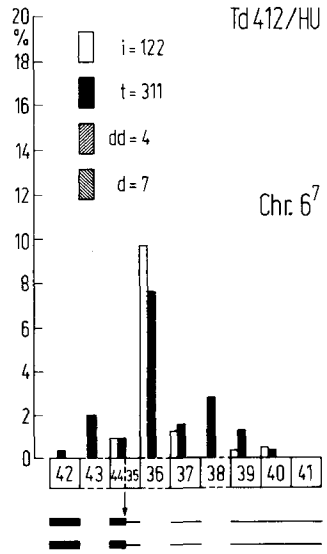

Fig. 8. Chromosome 6^7 of translocation karyotype Td 412. NOR and satellite of chromosome 7 (segments 43 and 42) are translocated from their original sites to satellite of chromosome 6, this resulting in two NORs combined tandemly

ment is no longer a translocation hot spot after treatment with HU of this karyotype (Fig. 8). The frequency of the translocations in segment 43 did not surpass the upper limit of confidence intervals (15).

Therefore, despite the fact that NORs of satellite chromosomes 7 (segment 43) in karyotypes T 505, T 506, Td 130 and Td 412 possess about 60-70% more rRNA cistrons than the NORs of chromosomes 6 (segment 36) (Subrahmanyam and Azad 1978) a clearly lower involvement in aberrations of this segment was observed after treatment with HU.

Conclusions

As is evident from the data presented in Figs. 1, 2, 3, the pattern of aberration distributions in the reconstructed barley karyotype MK 14/2034, the standard karyotype and the translocated karyotype T 21 provide clear evidence of preferential involvement in HU-induced aberrations of chromosome segments 36 and 43 (NORs) of satellite chromosomes 6 and 7. As a result of this, the percentage involvement in aberrations of segments 36 and 43 ranges from 34.2 to 41.7% of the total involvement in aberrations of all chromosome segments in the karyotypes.

These facts are consistent with and confirm the observations of Kihlman et al. (1966): in *Vicia faba* cells, treated with HU, a high proportion of the chromatid exchanges obtained were localized in the nucleolar constriction, i.e. the nucleolus organizer regions. Furthermore, our result demonstrate that there are clear differences of mutagen sensitivity between NOR 6 and NOR 7 localized at their original (standard) sites, since it was invariably shown that NOR 7 (segment 43) displayed a higher frequency of breaks and translocations than NOR 6 (segment 36) in the three karyotypes analyzed. This means that NORs 6 and 7 in standard positions respond somewhat differently to treatment with HU and aberration induction, respectively. At present, no completely satisfying explanation of this extremely high involvement of barley NORs is available, but it seems certain that breaks resulting in translocations occur inside the secondary constrictions, i.e. these exchange events evidently involve the ribosmal genes (rRNA and spacers) of the NORs.

If it is true that the NOR of satellite chromosome 6 in barley is functionally stronger in the organizing nucleoli than the NOR of satellite chromosome 7 (Tsuchiya 1952; Suzuki 1959), or that the expression and size of the secondary constriction would be a reflection of the extent of dispersion of rDNA (Doershug 1976; Givens and Phyllips 1976), then the higher aberration frequencies observed in NOR 7 violates the expectations that mutagen sensitivity could be connected with the functional activity of the NORs or the higher extent of dispersion of rDNA, respectively.

As it is evident by the observations made on *Hordeum vulgare* (Subrahmanyam and Azad 1978), there is an additive but unequal contribution of the two pairs of satellite chromosomes 6 and 7 in terms of number of rRNA cistrons. The unexpected feature concerning this observation was that the number of rRNA cistrons per NOR in satellite chromosomes 6 and 7 of barley karyotype is 1.588 and 2.698 rRNA (18S + 5,8S + 26S) cistrons respectively, i.e. the NOR of satellite chromosome 7 carries about 60-70% more rRNA cistrons than NOR of satellite chromosome 6 (Subrahmanyam and Azad 1978).

Since the NORs of chromosome 7 (segment 43) in standard positions show a higher involvement in aberrations than NORs of chromosome 6 (segment 36), it appears that the mutation sensitivity in terms of chromosome structural changes induced by HU depends somewhat on the number of rRNA cistrons per NOR. In addition to this, however, the results presented in this paper endorse a correlation between the synthetic activity (transcription) of NORs and the frequencies of chromosome mutations induced.

Experimental evidence in favour of that comes from the different translocation barley lines studied here. It has already been previously reported (Nicoloff et al. 1977, 1979; Anastassova-Kristeva et al. 1977, 1979) that appropriate structural changes of barley chromosomes may result in modifications of the normal pattern of nucleolus formation. The standard karyotype of barley consists of 7 chromosome pairs, 2 pairs of which (chromosome pairs 6 and 7) are satellite chromosomes. Transcription of rDNA located in the NORs of the four barley satellite chromosomes results in the formation of a maximum number of 4 primary nucleoli (10μm in diameter) at late telophase of mitosis. The maximum number of 4 nucleoli per nucleolus, i.e. the 'primary' number (Heitz 1931) corresponds to the number of nucleoli expected from the presence of two nucleolar satellite chromosome pairs with 4 NORs.

Due to chromosome structural changes, NORs may become translocated from their original sites which results in certain cases in modifications of synthetic activity (transcription) of rRNA cistrons in NORs, or, respectively, in the normal formation and morphology of nucleoli. Thus combination by means of translocation of all four NORs into a single chromosome pair either in opposite arms (karyotypes T 505 and T 506) or tandemly in one chromosome arm (karyotypes Td 130 and Td 412) of chromosomes 6 or 7 was found to result in complete or partial repression of the rRNA cistrons. This phenomenon has already been deduced from the impaired nucleolus formation by two of the NORs (Nicoloff et al. 1977, 1979; Anastassova-Kristeva et al. 1977, 1979).

Thus, in translocation lines T 505 and T 506, with all four NORs localized in opposite arms of chromosome 7 (7^6 – T 505) or 6 (6^7 – T 506), the maximum number of primary nucleoli per telophase nucleus is two nucleoli of standard size (about 10 μm in diameter) and two micronucleoli (about 1 μm in diameter) (Nicoloff et al. 1977, 1979). The two micronucleoli are indicative of impaired rDNA transcription from two of these NORs.

The same effect, i.e. suppression of synthetic activity of NORs was confirmed when the NORs were tandemly arranged in the same chromosome arm in karyotype Td 130 and Td 412, since the maximum number of primary nucleoli was also two nucleoli of standard size (7–10

μm in diameter) and two micronucleoli (about 1 μm in diameter). It has been suggested that the stronger NOR 6 is the one which forms the larger nucleoli while NOR 7 remains suppressed in all cases of NOR combination by interchange in a single chromosome pair (Anastassova-Kristeva et al. 1979, 1980). These observations provide additional evidence for the rule previously postulated: in reconstructed barley chromosomes with two NORs, only one is fully active (Nicoloff et al. 1977, 1979). Though the mechanism underlying this phenomenon is presently unknown, the cases represent what may be called position effects with respect to the transcription of rDNA.

Reconstructed barley karyotype (T 21) with three instead of two chromosome pairs with nucleolus organizing activity, due to interchanges with one translocation point inside the NOR, revealed quantitative relationships between the size of the secondary constriction (the amount of rRNA cistrons) and the number and size of the nucleoli being formed. Although the number of rRNA cistrons in NOR of satellite chromosome 7^5 (segment 43^{30}) was reduced in this karyotype when compared to the standard karyotype, no suppression of NOR activity was observed. The maximum number of primary nucleoli per nucleus was 6: two of standard size (produced by NORs of chromosome pair 6), two somewhat smaller ones (from NORs of chromosome pair 7^5), and two micronucleoli (produced by NORs of chromosome pair 5^7) (Anastassova-Kristeva et al. 1977; Nicoloff et al. 1977).

The merits of these translocation karyotypes are that in all of them (with the exception of karyotype T 21) the original number of rRNA cistrons in NORs 6 and 7 remains unchanged but due to position effects subsequent to transpositions of NORs, the synthetic activity of the rRNA cistrons is altered.

According to the data obtained in this study of karyotype T 505, where segment 43 (NOR of chromosome 7) occupies its original position in translocation chromosome 7^6, the aberration frequency decreased threefold as compared with the standard karyotype. Moreover, in karyotype T 506 (where segment 43 is transposed from its original position) the aberration frequency is much lower. In both karyotypes the aberration frequencies in segment 36 (NORs of chromosome 6) remained approximately the same as in the standard karyotypes.

The same also holds true for karyotypes Td 130 and Td 412. In both karyotypes the involvement in aberrations of segment 43 is practically insignificant, while the aberration frequencies in segment 36 either remain the same (karyotype Td 412 – segment 36 in its original site) or increase significantly (karyotype Td 130 – segment 36 transported from its original position).

From the present results it may therefore be assumed that the aberration frequency and mutagen sensitivity of NORs (segments 36 and 43) is correlated to the synthetic activity (transcription) of the rRNA cistrons in reconstructed chromosomes 7^6 and 6^7.

Whatever the mechanism(s) involved in this phenomenon, the present data indicate a close connection between the different rate of synthetic activity in nucleolus formation, and the range of variation in aberration involvement of NORs of chromosomes 6 and 7 (segments 36 and 43). This may be due to structural characteristics correlated with the different degrees of transcriptional activity of these NORs.

Studying the oocytes of a heterozygous mutant of *Xenopus laevis* with only half of the standard (wild) type number of rRNA genes, Reeves (1976) presented evidence that actively transcribing rDNA genes in the nucleolus are not in the state of usual protected nucleosome type structure.

That the ribosomal genes are not folded into nucleosomes but are in the extended B form of DNA has been also suggested by Scheer et al. (1977) after measurements of the transcription regions in actively transcribing chromatin.

Franke et al. (1978) have shown that regions of transcription appear after dispersion and spreading as 'axial intercepts' covered with lateral RNP fibrils and separated by regions, which are usually free of lateral fibrils and may represent non-transcribed spacers.

Studying transcribing rDNA chromatin in the electron microscope, Grainger and Ogle (1978) have found that the non-transcribed spacer region has a beaded structure, and by comparative measurements of non-transcribed and transcribed regions they suggested that the transcribed region of the rDNA is fully extended during transcription.

Corresponding results from electron microscopic studies on systems possessing different transcriptional activity were also obtained by Scheer et al. (1978).

These findings and our results are consistent with the approach to attribute significance to the decondensation-condensation cycle of chromatin in the transformation of DNA lesions into aberrations at the chromosome level, as argued by Kaina (1979). It was suggested that differential premitotic chromatin condensation may play a decisive role in the organization of chromosome structural changes by differentially influencing the fate of DNA lesions. Based on data from the literature, Kaina (1979) arrived at interesting deductions, one of which is that 'breaks and exchanges at the DNA level in non-permanently condensed chromatin regions, i.e. in functionally active euchromatin, will, with higher probability, become transformed into breaks and exchanges as observed in mitotic chromosomes than those induced in permanently condensed chromosome regions (PCR), i.e. heterochromatin and functionally inactive euchromatin.' Aberrations should therefore be located almost exclusively within actively transcribing euchromatic chromatin regions.

This conclusion gains support from the experiments with standard and translocation barley lines presented in this paper. Thus, it is highly probable that in standard karyotypes in the NOR of chromosome 7 (which contains about 70% more rRNA cistrons) a larger fraction of these cistrons is active in transcription, though with less intensity and vice versa, in NOR of chromosome 6, i.e. the rate of transcription in either of the NORs is different and they respond differently to aberration induction.

There is also further evidence that in the barley translocation lines analyzed, in which, due to position effects,

the synthetic activity (transcription) of NORs is altered, rDNA in NORs of chromosomes 6 and 7 goes through a cycle of a condensation and extension as a function of the specific transcriptional activity, and aberration induction in these NORs is coupled not with the condensed (beaded, nucleosomal) state but with the extended (transcriptional) state.

Therefore, based on the present results, there are some lines of evidence which indicate dependence of chromatid aberration induction upon transcriptional activity of rDNA in barley.

Although other explanations are possible, the present results indicate a likely and a pertinent direction for further researches.

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